isoform specific nature of kinesin inhibition by Tau and its lack of inhibition on microtubules in the GTP-nucleotide state.

1656-Pos Board B548
Kinesin-2 Navigates Microtubule Obstacles more Efficiently than Kinesin-1
Gregory Hoeprich[1], Tony Jiang[1], William Hancock[2], Christopher Berger[1].
[1]University of Vermont, Burlington, VT, USA, [2]The Pennsylvania State University, University Park, PA, USA.
During axonal transport, an ensemble of molecular motors, including kinesin-1 and kinesin-2, navigate a complex microtubule landscape to deliver cargo to their target destinations within the cell. It has previously been shown *in vitro* that the neuronal microtubule associated proteins, 3RS-tau and 4RL-tau, reduce kinesin-1 processivity on taxol-stabilized GDP microtubules, but not on microtubules stabilized with GMPCPP (a slowly hydrolyzable GTP analog). Furthermore, kinesin-1 processivity is also reduced on GMPCPP microtubules relative to taxol-stabilized microtubules, suggesting the microtubule lattice modulates interactions with both kinesin-1 and tau (McVicker *et al.,* (2011) *J Biol Chem* 286:42873). However, the effects of tau and the microtubule lattice structure on kinesin-2 processivity are still unknown. Kinesin-2 is known to have a longer neck-linker than kinesin-1, resulting in reduced coordination between motor domains and decreased processivity on taxol-stabilized microtubules (Shastry *et al*., (2010) *Curr Biol* 20:939). We hypothesize that these differences in kinesin-2 function make it less sensitive to alterations in the microtubule lattice than kinesin-1, and allow it to more easily navigate obstacles, such as tau, on the microtubule surface. To directly test this hypothesis, we used single molecule imaging with TIRF microscopy to measure kinesin-2 motility as it stepped along microtubules in different nucleotide states (GDP or GMPCPP) in the absence or presence of 3RS-tau and 4RL-tau. Our results demonstrate that, in contrast to kinesin-1, kinesin-2 processivity is unchanged on taxol-stabilized vs. GMPCPP microtubules and is insensitive to the presence of either 3RS-tau or 4RL-tau. Thus, while kinesin-2 is less processive than kinesin-1, it may be better optimized to navigate around obstacles on different microtubule lattice structures, allowing the two motors to work together for the efficient delivery of cargo in the complex environment of the neuronal axon.

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Human Disease-Related Mutation at R262 of β3-Tubulin Critical for Kinesin Motility and ATP Hydrolysis
Yoshihiko Yamakita, You Hachikubo, Rie Ayukawa, Seiichi Uchimura, Etsuko Muto, **Itsushi Minoura**.
RIKEN BSI, Wako, Japan.
Kinesin-microtubule interaction plays essential roles in brain development and function. In human neuronal cells, a point mutation, R262C/H, in β3-tubulin causes a disrupton in the interaction of microtubules with kinesin, leading to developmental disorders collectively termed TUBB3 syndrome (Tischfield, 2010). To clarify the molecular mechanism underlying the disease, in vitro functional analysis of mutant microtubules is essential. Aiming to examine the effect of tubulin mutation on kinesin motility and ATPase activity, here we developed a method to express and purify recombinant human tubulin using a baculovirus-insect-cell expression system. The method yields >1 mg of human α1β3-tubulin with a purity of >95% from 1 L culture of High Five cell. In a single-molecule motility assay, while kinesin moved along wild type (WT) human microtubules at a velocity comparable to that on porcine brain microtubules, it scarcely bound to β3-R262A microtubules. In parallel with this observation, in β-R262A mutants, the maximum ATPase rate (k_{cat}) was reduced four-fold and the Michaelis constant ($k_{M,MT}$) was 40 times larger than that of WT. These results clearly demonstrate the involvement of β-R262 in kinesin-microtubule interaction. Compared with other critical residues previously identified using yeast mutant microtubules (Uchimura 2010), β-R262 is unusual because it is the only positively charged residue critical for kinesin-microtubule interaction. Whether the β-R262 residue directly binds to a putatively negatively charged residue in kinesin or modulates kinesin-microtubule interaction through repulsion will be discussed at the poster session. Our new method provides a powerful tool to perform molecular level analyses on the function of tubulin/microtubules associated with human diseases.

1658-Pos Board B550
Protein Friction causes Molecular Wear in Kinesin-Powered Molecular Shuttles
Emmanuel Dumont, Henry Hess.
Columbia University, New York, NY, USA.
During the operation of biological nanomachines, such as motor proteins, energy has to be dissipated to enable cycles of unbinding and rebinding leading to stepwise movement. Since the energy dissipation is, for low velocities, proportional to the velocity of movement, it can be conceptually understood as ''protein friction''. At the same time, the forces generated during the unbinding steps can be expected to generate adhesive wear. Here, the degradation of microtubules gliding on surfaces covered by kinesin motor proteins is examined, a situation with relevance to biological systems as well as engineered hybrid systems, such as molecular shuttles powered by motor proteins. We find that the gliding motion leads to a detectable shortening of the microtubules that depends non-linearly on the kinesin density and the microtubule velocity. We interpret our data as a result of the sequential removal of the most weakly bound tubulin dimers from the end of the microtubule by the motors.

1659-Pos Board B551
Dissecting the Mechanisms of Katanin-Mediated Microtubule Severing and Depolymerization
Megan E. Bailey, Margaret M. Morelli, J. Daniel Diaz, Jennifer L. Ross.
University of Massachusetts at Amherst, Amherst, MA, USA.
Microtubules are important for forming networks in cells from mitotic spindles and cilia to neuronal networks. Microtubule networks are regulated by a variety of proteins including microtubule-severing enzymes, which regulate the length of microtubule filaments through severing and depolymerization. Katanin, the first-discovered microtubule severing enzyme, is a AAA+ enzyme that oligomerizes into hexamers and uses ATP hydrolysis to sever microtubules. It has been suggested that katanin targets to microtubule defects, and we showed that katanin targets interfaces between microtubules made with dislocation defects. To further dissect the severing and depolymerization abilities of katanin, we performed in vitro biophysical assays using four different types of microtubules with different known defects to distinguish where katanin may be targeting. Our results show that there is preferential severing of microtubules with fewer protofilaments and that the c-terminal tail is required for severing activity. However, microtubules without any c-terminal tails are still depolymerized and do so at a faster rate, suggesting that katanin utilizes two different mechanisms to drive depolymerization and severing of microtubules.

1660-Pos Board B552
Characterization of Kinesin-1 E157A and E157K Mutants
Leslie Conway, Jennifer L. Ross.
University of Massachusetts Amherst, Amherst, MA, USA.
Kinesin-1 is a microtubule-based cargo-transporting motor that plays an important role in axonal transport. Kinesin is responsible for bringing newly synthesized materials from the cell body to the axon terminal in order to properly maintain long axonal processes. Numerous mutations in the kinesin-1 gene have been found to affect the ability of these motors to transport vital material to the axon terminal, resulting in neuronal degeneration. Kinesin possesses two motor domains, each of which contains an ATP binding pocket as well as a microtubule-binding domain. Here we study two mutations in the human kinesin gene, E157A and E157K, shown to inhibit development in flies. This residue position is located near the microtubule-binding site on the motor domain. We find that, in a filament gliding assay, microtubule gliding velocities are reduced for kinesin E157A and E157K mutants compared to wild type kinesin. Interestingly, single molecule studies using these same mutants do not show a reduced velocity compared to wild type. Single molecule studies also reveal that while these mutants have velocities identical to wild type kinesin, they show reduced run lengths. The difference in these two assays is that kinesin motors must coordinate in the filament gliding assay, but not in the single-molecule assays. All together, these results suggest that in addition to a defect in processivity, these mutants possess a cooperativity defect, resulting in slow motility only when groups of mutant motors must work together. These results are significant because, in the cell, multiple motors transport cargos, so defects in coordination between motors are a unique mechanism to inhibit transport.

1661-Pos Board B553
Direct Observation of the Forward Stepping Motion of Kinesin-1 using Dark-Field Microscopy with 50-Micro Second Temporal Resolution
Hiroshi Isojima[1], Ryota Iino[2], Hiroyuki Noji[2], Michio Tomishige[1].
[1]Department of Applied Physics, The University of Tokyo, Tokyo, Japan, [2]Department of Applied Chemistry, The University of Tokyo, Tokyo, Japan.
Kinesin-1 moves along microtubule by alternately moving two motor domains, in which the trailing head detaches and displaces toward the 16-nm forward tubulin-binding site upon ATP binding to the partner head. However, the detailed process of the forward stepping motion is still unclear, mainly due to the lack of temporal resolution of the single-molecule measurements. Here we employed dark-field microscopy with perforated mirror, and observed the movement of one of kinesin heads labeled with 40-nm gold particle with ~2 nm spatial and ~55 μs temporal resolutions. In the presence of saturating ATP, the gold particles showed ~16 nm discrete steps toward the microtubule long axis, which is consistent with the previous observations. However, our